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# Ameliorative Effect of Commiphora Myrrha Against Oxidative Stress Induced by High Fat Diet in Rats.

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## ABSTRACT

Obesity is a cause of health problems due to its strong association to many disorders' incidence as renal disease. This paper aims at estimating the evolution of oxidative stress in response to high fat diet (HFD) and the effect of Commiphora Myrrha resin ethanolic extract (CMEE) on renal MDA, glutathione reductase (GR) activity in kidney and histopathological alterations in renal tissue. Fifty adult Wister albino male rats were weighed from 100-120 g and assigned into five groups each of 10 rats. Rats were fed on a basal diet for 14 weeks (Cr group). Rats were fed on a basal diet and administrated orally CMEE at dose of 500 mg/kg for 14 weeks (CM group). Rats were fed on HFD for 14 weeks (HFD group). Rats were fed on HFD and orally administration of CMEE at dose of 500 mg/kg for 14 weeks (HFD + CM group). Rats were fed on HFD for 8 weeks then stop HFD and fed on a basal diet with oral administration of CMEE at dose of 500 mg/kg bodyweight for another 6 weeks (HFD then normal diet +CM group). MDA level and GR activity were estimated with histopathological examination of renal tissue of rats. A significant increase in MDA level and a significant decrease of GR activity in the HFD rats. Administration of CMEE to HFD rats in groups IV and V decreased significantly MDA level and increased significantly activity of GR enzyme as compared with the HFD group. Moreover, the GR activity reached to normal level in both groups compared in the control group. HFD induced obesity correlated to kidney disorders. CME had the ability to improve oxidative stress induced by HFD.

**Keywords:** *Obesity, Commiphora Myrrha, MDA, and GR.*

## INTRODUCTION

Obesity is a pathological condition leading to many adverse effects on health, due to accumulation of excess fat in body (Haslam and James, 2005). Oxidative stress increased in obese individual due to hyperglycemia, inadequate antioxidant defenses, increase of tissue lipid, increase of free radical formation, and chronic inflammation (Vincent and Taylor, 2006). Obesity affected many organs in the body such as liver, heart and kidney. Fatty liver and nephropathy are commons complication of obesity (Xavier and Sunyer, 2002). It is known that free radicals have adverse effect on cell survival due to membrane damage through the oxidative damage of lipid, protein and irreversible DNA modification (Mishra, 2004). Lipid peroxidation such as hydroperoxides levels and thiobarbituric acid reactive substances are markers of oxidative damage (Uzun *et al.*, 2007). In addition, oxidative damage is enhanced by the decrease in antioxidant enzymes activities such as glutathione reductase which acts as a free radical scavenger in conditions associated with oxidative stress (Blokhina *et al.*, 2002).

Medicinal plants used in folk medicine contain a wide range of substances that can prevent and treat many diseases. The medical value of these plants is due to presence of some bioactive constituents as, phytosterols, diterpenes, triterpenes and polyphenolic compounds (Edeoga *et al.*, 2005 and Noor *et al.*, 2013).

Commiphora Myrrha (family *Burseraceae*) is a small tropical plant tree that grows in East Africa and India (Haffor, 2010). It is a resinous exudate obtained from the stem of the plant's trees. Myrrha has been approved in USA by FDA as a safe flavoring agent in beverages and foods and as fragrance in cosmetics (Shen *et al.*, 2012). Chemical constituents of *Commiphora* includes monoterpenes (limonene, p-cymene, α-terpineol) and sesquiterpenes (β-bisabolene) (Demissew, 1993). Only some steroid components have been purified including Z and E guggulsterones, responsible for hypolipidemic activity (Urizar and Moore, 2003). The antioxidant effect of CM might be attributed to the presence of phenolic and flavonoid contents (Mahboubi and Kashani, 2016 ) besides limonene (plant component which is a monoterpene ) can restore the normal levels of reduced glutathione, glutathione peroxidase, glutathione reductase, glutathione S-transferase, catalase and malondialdehyde in mouse administrated 12-O-tetradecanoylphorbol-13-acetate to promote skin tumor development (Chaudhary *et al.*, 2012). This study has been carried out to investigate the oxidative stress markers histopathological alterations in the kidney in response to HFD and to estimate the effect of Commiphora Myrrha on HFD induced oxidative stress.

## MATERIAL AND METHODS

***Commiphora Myrrha:***

Commiphora Myrrha, family Burseraceae, was purchased from Abo-Al Maged herpes shop in Alexandria, Egypt. Myrrha resin presents in the form of yellowish masses. It has a bitter taste and an aromatic odour.

***Animals*:**

50 adult Wister albino male rats were purchased from animal house in from laboratory animal colony Cairo, Egypt weighing 100-120 g. All animals were housed in wide plastic cages under hygienic environment provided with balanced ration and clean water ad libitum in addition natural ventilation with 12 hours light/ dark cycle. Rats were kept for two weeks for acclimatization before beginning the experiment.

**Biochemical kits:**

Biochemical kits for estimation of malondialdehyde level and glutathione reductase activity were purchased from bio-diagnostic company Cairo, Egypt.

***Preparation of diet:***

**A) Basal diet:**

Control group of rats were fed on a basal ration purchased from Al- Wady Company. A basal diet composed of yellow corn, soybean seeds, soybean oil, limestones, monocalcium phosphate, sodium chloride, sodium bicarbonate, lecithin, mixture of minerals and vitamins (Table.1).

**B) High-fat diet (HFD):**

HFD was prepared according to Abd Eldaim *et al.* (2018). It is composed of 7% beef tallow, 8.3% yolk, 18.7% sucrose, and 66% standard diet.

***Preparation of Commiphora Myrrha resin ethanolic extract:***

After grinding of plant into a fine powder, 800 g of plant powder were soaked in 90% ethanol and kept in a fridge with daily shaking for 3 days. Ethanolic extract was filtered by using filter paper to remove any debris. The filterate was concentrated under pressure by rotary evaporator at 50 $^{o}$C. The semisolid Commiphora Myrrha ethanolic extract was kept in a refrigerator till further use. This procedure was described by (Shalaby and Hamowieh, 2010).

***Experimental design:***

A total of 50 male albino rats were randomly assigned into five groups each of 10 rats in this experiment which continued for 14 weeks:

**Group I (control):** Rats were fed on a basal diet for 14 weeks.

**Group II (CM):** Rats were fed on a basal diet and administrated CME by oral gastric tube at dose of 500 mg/kg bodyweight for 14 weeks according to Shalaby and Hammouda (2014).

**Group III (HFD):** Rats were fed on HFD for 14 weeks.

**Group IV (HFD+CM):** Rats were fed on HFD and orally administration of CME by oral gastric tube at dose of 500 mg/kg for 14 weeks.

**Group V (HFD then normal diet + CM):** Rats were fed on HFD for 8 weeks then HFD was stopped and fed on a basal diet with oral administration of CME at dose of 500 mg/kg for another 6 weeks.

***Tissue sampling:***

At the end of experiment (14 weeks) rats were scarified, kidneys were removed and weighed then washed in saline. Parts of kidneys were kept in 10% formalin for histopathological

studies. Other parts were preserved at -80 °C for MDA and GR investigations.

***Methods:***

A- **Biochemical analysis of tissue:** Determination of MDA content by (Satoh, 1978) technique and GR activity by (Cariberg and Mannervik, 1975) method were performed in kidney homogenate by using bio diagnostic kits. Prior to dissection tissue were perfused with phosphate buffer saline pH 7.4 to remove any red blood cells. 222 mg of tissues were homogenized in 5 or 10 ml cold buffer. Tissue homogenate was centrifuged at 4000 rpm for 15 minute. The supernatant was separated and used for determination of MDA and GR levels.

B- Histopathological examination of renal tissue according to Bancroft *et al.* (1996).

***Statistical analysis:***

Results were expressed as mean ± SE. Data were analyzed for significant difference by ANOVA way according to Snedecor and Cochran (1967). The statistical analysis was carried out using SPSS (Statistical package for Social Sciences) Version 16 released in 2007.

## RESULTS

***Lipid peroxidation (MDA) and glutathione reductase (GR) assessments:***

***CM modulates HFD increased MDA content and increased the lowered glutathione reductase activity in kidney of HFD rats after 14 weeks:*** Table (2) and fig. (1&2) showed that HFD consumption increased significantly MDA level and decreased significantly glutathione reductase activity in renal tissue of HFD rats in comparison with the control group. Administration of CM to HFD group in group IV (HFD + CM) and group V (HFD then normal diet + CM) decreased significantly MDA level and increased significantly glutathione reductase activity in renal tissue of both groups in comparison with the HFD group. Moreover, glutathione reductase activity in renal tissue of group IV and V attained its normal range as compared with the control group.

***Histopathological changes in renal tissue after 14 weeks:***

***Commiphora myrrha modulates the HFD histopathological alterations in renal tissue in different groups of rats*:** Microscopical examination of kidney specimens from different groups of rats showed normal renal tissue in group I (control) and group II (CM) (Fig. 3: A & B respectively). Kidney tissue from group III (HFD) showed congested glomerular capillary, interstitial inflammatory cells infiltration and perivascular edema, swelling of tubular epithelium and renal cast (Fig. 3: C). Renal tissues from group IV (HFD+ CM) showed slight congestion of glomerular capillary and mild swelling of tubular epithelium (Fig. 3: D). Examination of renal tissue from group V (HFD then normal diet+ CM) showed congested blood vessels, swelling of tubular epithelium with renal cast (Fig. 3: E).

**Table (1): Chemical composition of basal diet:**

| Chemical ingredients | (g %) |
|---|---|
| **protein** | 17% |
| **CHO** | 68.16% |
| **fat** | 4.9% |
| **Choline chloride** | 2% |
| **Vitamin mixture** | 1% |
| **Salt mixture** | 3.5% |
| **fiber** | 3.44% |

**Table (2): Renal MDA content (nmol/gm of tissue) and glutathione reductase (U/L) activity after 14 weeks:**

| Groups | I (Control) (14 W) | II (C M) (14 W) | III (HFD) (14 W) | IV (HFD+C.M) (14 W) | V (HFD, 8w) then (Basal Diet + C M 6W) |
|---|---|---|---|---|---|
| MDA (nmol/g) | $11.24\pm0.58^{c}$ | $11.94\pm0.9^{c}$ | $23.46\pm1.07^{a}$ | $17.20\pm0.54^{b}$ | $16.57\pm0.7^{b}$ |
| GR (U/L) | $8.03\pm1.80^{a}$ | $8.04\pm1.27^{a}$ | $1.61\pm0.98^{b}$ | $7.23\pm1.50^{a}$ | $6.43\pm0.98^{a}$ |

-Values are expressed as means ± SE (standard errors).

-The mean difference is significant at $P < 0.05$.
-Values carrying different letters in the same row have significant differences.
- MDA: malondialdhyde,; GR: glutathione reductase,.

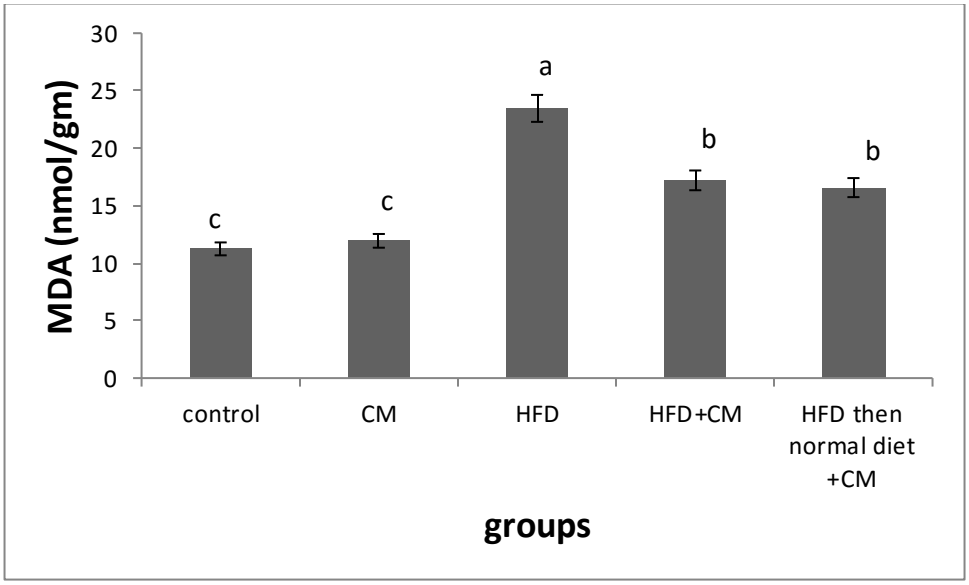


Figure (1): Renal MDA concentration (nmol/g) after 14 weeks in different groups of rats

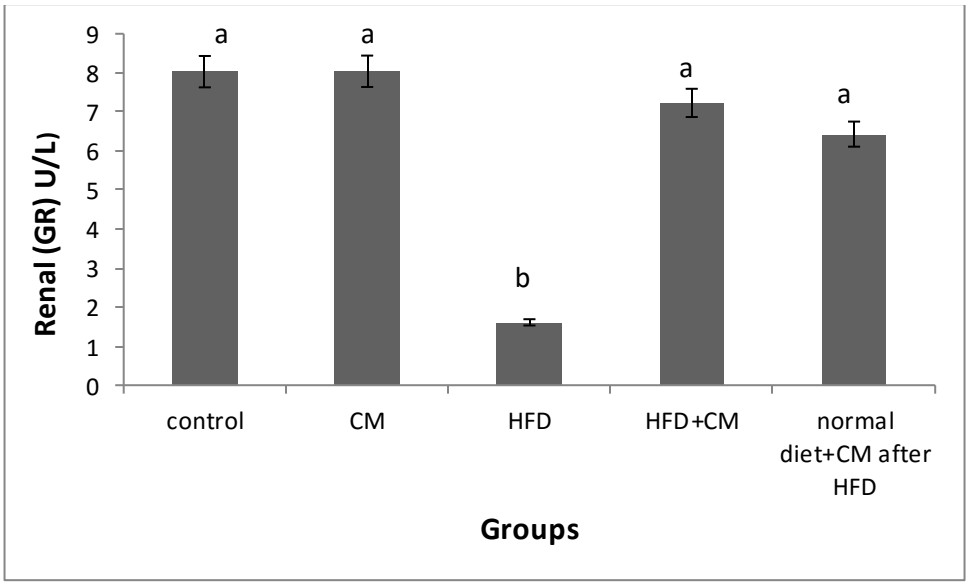


Figure (2): Renal GR activity after 14 weeks (U/L) in different groups of rats.

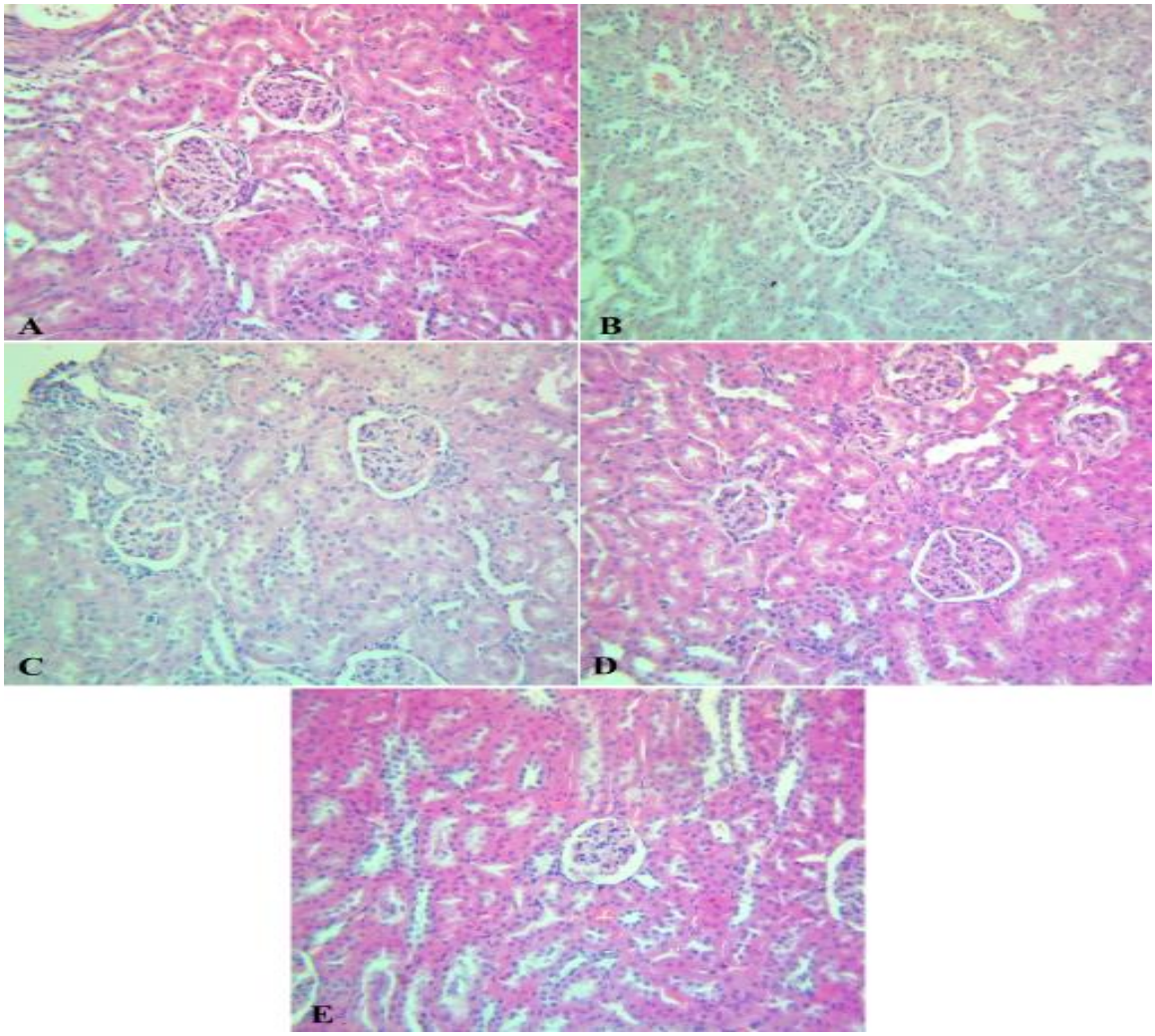


Figure 3: Histopathological alterations in kidney of different groups of rats: A & B. Renal tissues from group I and II respectively; C. renal tissue from group III; D. renal tissue from group 4; E. renal tissue from group 5. (H&E X40).

**DISCUSSION**:

Obesity and dyslipidemia are metabolic disorders represent a serious health problem that increased risk of many diseases such as cardiovascular disease, diabetes mellitus, hypertension and oxidative stress (Afolayan and Mbaebie, 2010). Treatment of obesity by chemical drugs had many adverse side effects as alterations in the brain lipids composition which in turn causes neurological and mental disorders due to administration of statins (Marek *et al.*, 2004). They stated that treatment of obesity by 0.1mg of fenofibrates, lovastatin, pravastatin or Fluvastatin once daily for six weeks had significant decrease in body weight but with many adverse side effects including neurological and mental symptoms in addition tissue damage (myopathy) and hepatomegaly. For these findings this paper researches for a safe and a cheap source for treatment of obesity. Commiphora Myrrha is a medicinal plant used as antioxidant, hypoglycemic, hypolipidemic and antidiabetic (Ramesh and Saralakumari, 2012). That's why the main object of this study is investigation the effect of Commiphora Myrrha to ameliorate HFD induced oxidative stress.

This study revealed that the HFD consumption induced oxidative stress in obese rat as shown in table (2) by a significant increase in MDA level and marked decrease in glutathione reductase activity. This enzyme catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) which serves as antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for glutathione peroxidases and glutathione s-transferases in organic peroxide detoxification and xenobiotics metabolism respectively (Lu, 2009). These findings were in agreement with (Noeman *et al.*, 2011) who stated that HFD consumption significantly increased MDA and protein carbonyl (PCO) levels in the hepatic, heart and renal tissues of obese rats, as well as a significant decrease in the activity of glutathione S- transferase (GSH), glutathione peroxidase (GPx) and paraoxonase1 (PON 1) enzymes.

In contrast, administration of CM to HFD in groups IV &V in current study showed a significant decrease MDA level and marked increase activity of glutathione reductase (GR). Moreover, GR of both groups reached to normal level as the control group. These findings were in agreement with Ramesh and Saralakumari, (2012) who stated that administration of Commiphora Mukul to fructose fed rate decreased significantly MDA level and increased significantly GR activity as CM prevented depletion of GR activity by maintaining normal level of this enzyme that revealed protection effect of C. mukul against oxidative damage through keeping normal GSH level. Also Salama *et al.*, (2014) supported our findings as they recorded a significant increase in total antioxidant capacity in kidneys of diabetic group treated with mirazid (Myrrha extract), due to various phytonutrients in Myrrha which act as good antioxidant and protect against oxidative damage to macromolecules. The antioxidant effect of CM might be attributed to the presence of phenolic and flavonoid contents (Mahboubi and Kashani, 2016 ) besides limonene (plant component which is a monoterpene ) (Demissew, 1993) that able to restore the level of reduced glutathione, glutathione peroxidase, glutathione reductase, glutathione S-transferase, catalase and malondialdehyde production in mouse administrated with 12-O-tetradecanoylphorbol-13-acetate to promote skin tumor development (Chaudhary *et al.*, 2012). Also, CM antioxidant effect or may be attributed to inhibition of eicosanoid generation, cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism which are potent modulators of lipid peroxidation (Salama *et al*., 2014). Other mechanism of Myrrha as its component (retinol, ascorbic acid & tocopherol) may be scavenging of peroxides and other radicals (Bond *et al*., 1993). Also, presence of selenium in *Myrrha* resin in amount (1.41ppm), plays a role in the antioxidant activities (Kieliszek and Błazejak, 2013) via certain enzymes, include thioredoxin reductase (TRxR), glutathione peroxidase (GPx) and deiodinase iodothyronine.

HFD consumption significantly affected on microscopical examination of rat kidney in HFD group as showed congested glomerular capillary, interstitial inflammatory cells infiltration and perivascular edema, swelling of tubular epithelium and renal cast (Fig. 3: C) as compared with control group. These findings were in the line with Noeman *et al.* (2011) who stated that the accumulation of lipid and lipid peroxidation around kidney of obese rats penetrates into the medullary sinuses thus enhanced intra-renal pressures that may cause damage the renal tissue. Damaged renal tissue acts as sources of ROS and develops lipid peroxidation. An increased lipid peroxidation in the kidney tissue, as well as modification of the circulating LDL/VLDL fraction, is probably involved in the onset of kidney lesions in this normoglycaemic rodent model of obesity. Also, Abd Eldaim *et al.* (2018) stated that consumption of HFD for 10 weeks showed diffuse and extensive micro vesicular steatosis with great enlargement and presence of intra cytoplasmic acidophilic globular body.

CM consumption reversed histopathological alterations in (HFD+CM) group and (normal diet + CM after HFD) group as compared with HFD group where renal tissues from group IV (HFD+ CM) showed slight congestion of glomerular capillary and mild swelling of tubular epithelium (Fig. 3: D). Examination of kidney tissue from group V (normal diet+ CM after HFD) showed congested blood vessels, swelling of tubular epithelium with renal cast (Fig. 3: E). This is in agreement with Ahmad *et al.*, (2015) who stated that rats treated with D-GalN/LPS and 500 mg/kg of CME showing the absence of inflammatory cells, ballooning, regeneration of hepatocytes around central vein but slight congestion in central vein and almost toward near normal liver architecture and possessing higher hepatoprotective action. This is attributed to the chemical constituent of CM by GC-MS (Gas chromatography- mass) which were curezerine, delta-elemene, beta-elemene, 3-[(E)-2-phenyl-1-propenyl] cyc, bicycle [3.1.1]hept-2-ene-2-car, 8-isopropenyl-9-isopropyltetra, and 2,4-bis(3-methyl-1-pentynyl)-4, that were stated to have cytoprotective, antioxidant and anticancer effects (Racine and Auffray, 2005; Fraternale *et al.*, 2011; Marcotullio *et al.*, 2011)

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